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REVIEW

Multiple Irregular Antibodies in Alloimmunized Patients: immunohematologic techniques for their detection and typing

Anticuerpos irregulares múltiples en paciente aloinmunizados: técnicas inmunohematológicas para su detección y tipificación

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ABSTRACT

Introduction: alloimmunization is a frequent complication in chronically transfused patients, resulting from repeated exposure to erythrocyte antigens that differ from the recipient's own. This immune response leads to the development of irregular alloantibodies that compromise transfusion compatibility and increase the risk of hemolytic reactions, particularly when multiple alloantibodies coexist.
Method: a systematic review was performed following the PRISMA guidelines, based on a search of regional and international scientific databases. Studies published between 2020 and 2025 that addressed alloimmunization, the presence of multiple irregular erythrocyte alloantibodies, and the immunohematologic techniques used for their detection were included.
Results: the most frequently identified alloantibodies primarily belonged to the Rh and Kell blood group systems, with recurrent simultaneous combinations among them. For their detection, the manual tube test, column agglutination (CAT), and solid-phase red cell adherence (SPRCA) techniques were evaluated. SPRCA demonstrated higher sensitivity for detecting weak or coexisting alloantibodies, whereas CAT provided standardized interpretation and broad clinical applicability.
Conclusions: the detection of multiple irregular alloantibodies requires a strategic combination of immunohematologic methodologies. Although SPRCA offers superior analytical sensitivity in complex samples, CAT remains a reliable and widely accessible alternative in routine clinical laboratory practice, supporting safer transfusion decision-making.

Keywords: Alloimmunization; Irregular Erythrocyte Antibodies; Blood Group Antigens; Coombs Test; Column Agglutination; Solid-Phase Adherence.

RESUMEN

Introducción: la aloinmunización constituye una complicación frecuente en pacientes politransfundidos, originada por la exposición repetida a antígenos eritrocitarios diferentes a los propios. Este proceso inmunológico desencadena la formación de anticuerpos irregulares que dificultan la compatibilidad transfusional y elevan el riesgo de reacciones hemolíticas cuando coexistieron múltiples aloanticuerpos.
Método: se realizó una revisión sistemática siguiendo el modelo PRISMA, basada en la búsqueda de literatura en bases de datos regionales e internacionales. Se incluyeron estudios publicados entre 2020 y 2025 que abordaron la aloinmunización, la presencia de anticuerpos eritrocitarios irregulares múltiples y las técnicas inmunohematológicas utilizadas para su detección.
Resultados: los anticuerpos descritos con mayor frecuencia correspondieron principalmente a los sistemas

eritrocitarios Rh y Kell, observándose combinaciones simultáneas entre ellos. Para su detección se analizaron la técnica en tubo, la aglutinación en columna (CAT) y la adherencia de eritrocitos en fase sólida (SPRCA). Este último mostró mayor sensibilidad para reconocer anticuerpos débiles o coexistentes, mientras que CAT proporcionó una lectura estandarizada con buena aplicabilidad clínica.
Conclusiones: la identificación de anticuerpos irregulares múltiples requirió la integración estratégica de diferentes técnicas inmunohematológicas. Aunque SPRCA ofreció mejor desempeño en muestras complejas, CAT se mantuvo como herramienta efectiva y accesible en la mayoría de laboratorios, favoreciendo decisiones transfusionales seguras.

Palabras clave: Isoinmunización; Anticuerpos de Grupo Sanguíneo; Antígenos de Grupo Sanguíneo; Prueba de Coombs; Pruebas de Aglutinación; Técnicas de Fase Sólida.

INTRODUCTION

Transfusion therapy is a fundamental component in the clinical management of patients with chronic anemia, hematologic malignancies, renal failure, or those undergoing highly complex surgical procedures, as it enables the safe and effective replacement of essential blood components.[1] However, repeated exposure to erythrocyte antigens that differ from those of the recipient may elicit a specific immune response, leading to alloimmunization, characterized by the production of irregular alloantibodies directed against donor red cell antigens.[2,3]

Alloimmunization represents a significant clinical challenge in hemotherapy, as it may lead to post-transfusion hemolytic reactions and complicate the identification of compatible blood units, thereby compromising transfusion safety.[4,5,6] Consequently, immunohematologic surveillance and the implementation of extended erythrocyte compatibility testing between donor and recipient are essential strategies to mitigate these risks.[6,7]

Most irregular alloantibodies are of the IgG class, warm-reactive, and high affinity, which makes them clinically significant due to their hemolytic potential and their ability to cross the placenta.[8,9] In contrast, IgM alloantibodies are less frequent and generally naturally occurring and cold-reactive, with limited clinical relevance since they do not react at 37°C. [9] The blood group systems most commonly implicated include Rh, Kell, Duffy, Kidd, and MNS, with *anti-E*, *anti-K*, and *anti-D* being particularly frequent and transfusion-relevant. The prevalence of these alloantibodies may exceed 30 % in chronically transfused patients.[10,11]

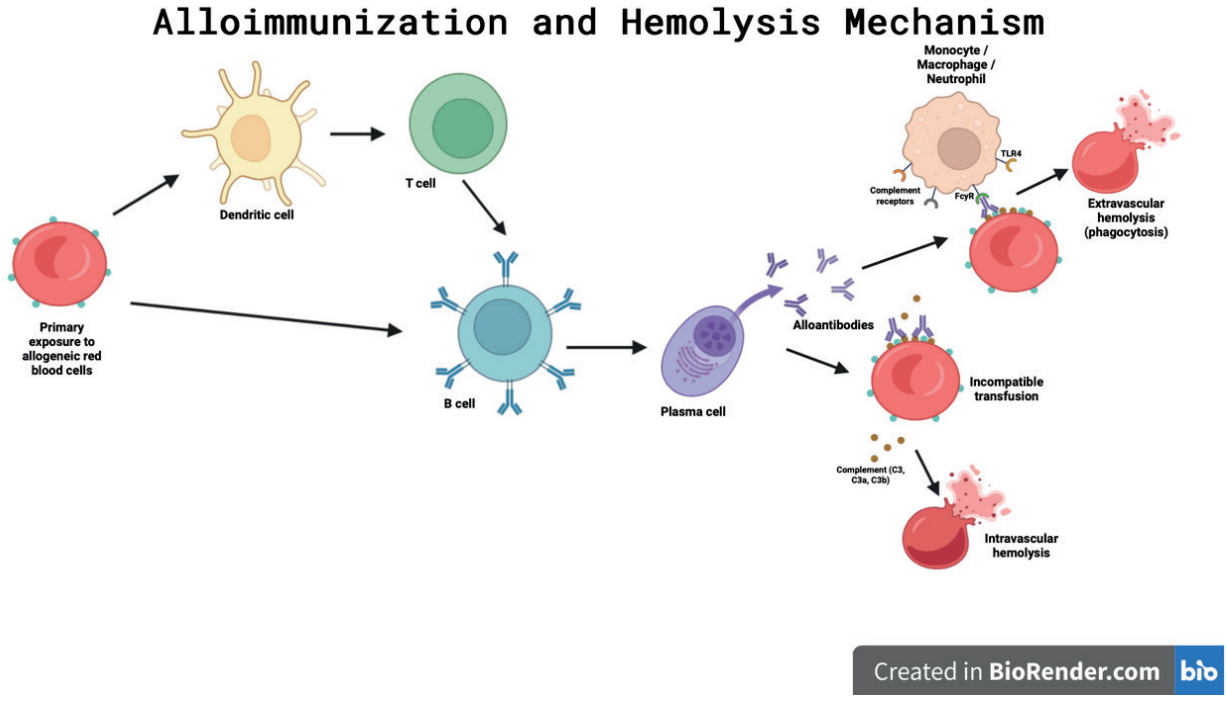

Figure 1. Alloimmunization and Hemolysis Mechanism

The detection of multiple irregular alloantibodies presents its greatest challenge in the phenomenon of antigenic masking, which may reduce serologic reactivity and complicate antibody identification.[12,13] Over time, immunohematologic methods have evolved toward more sensitive and standardized approaches, including

column agglutination (CAT) and solid-phase red cell adherence (SPRCA), both of which improve analytical reproducibility and facilitate the detection of weak-titer or low-affinity alloantibodies.[14]

Therefore, this study evaluates the diagnostic performance of immunohematologic techniques used for the detection and characterization of multiple alloantibodies, with the objective of strengthening transfusion safety and ensuring high-quality compatibility practices.

METHOD

The literature review was conducted in accordance with the PRISMA guidelines (figure 2). Independent searches were carried out in English-language databases (PubMed, Web of Science, ScienceDirect, and the Virtual Health Library) and in the Spanish-language database SciELO. Studies published between 2020 and 2025 were identified using controlled vocabulary (MeSH) and free-text keywords. The following Boolean search strategy was applied:(Isoantibodies OR Alloimmunization) AND (Blood Group Antigens OR Erythrocytes) AND (Blood Transfusion) AND (Clinical Laboratory Techniques OR Agglutination Tests OR Coombs Test OR Immunohematology Tests).

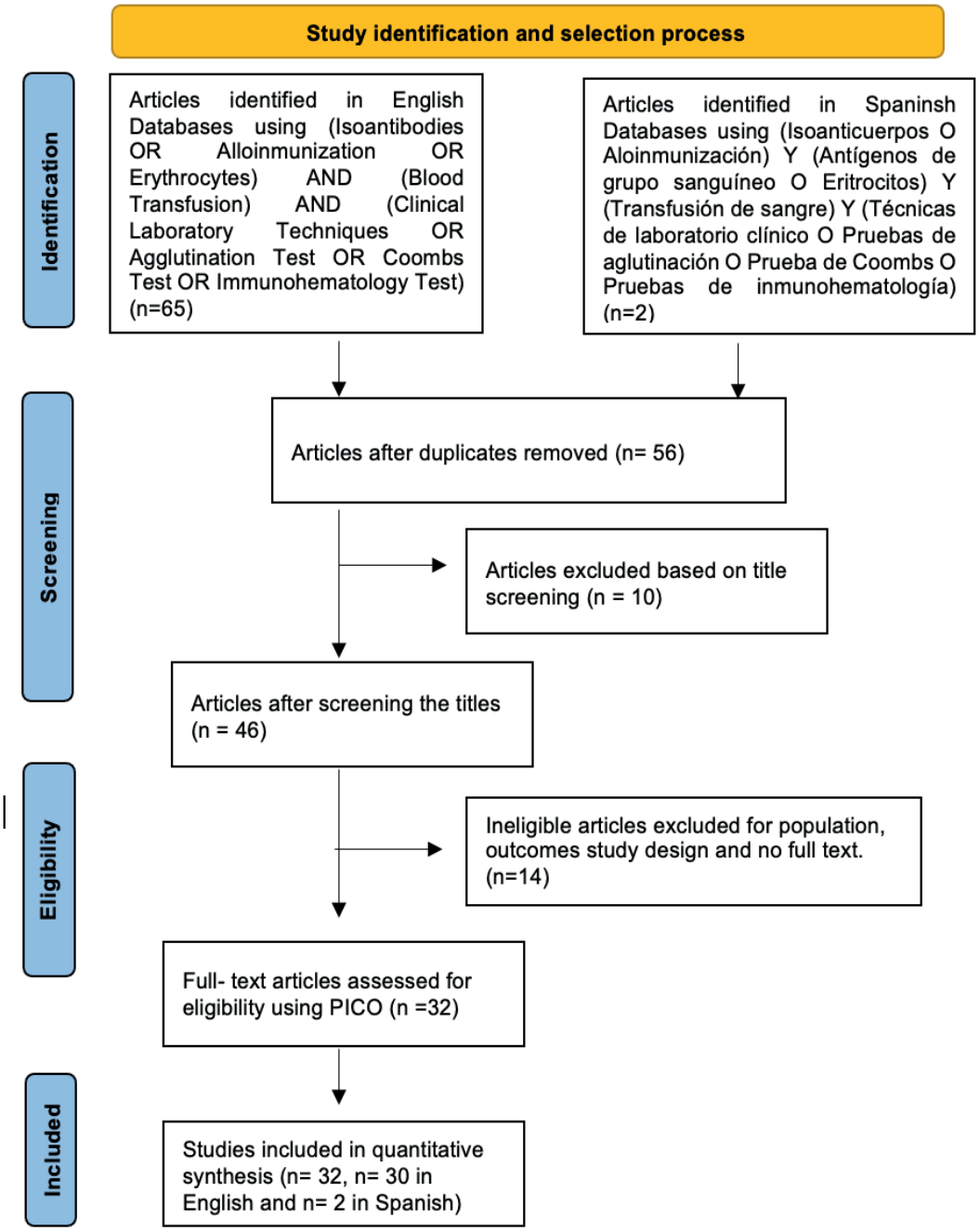

Figure 2. Diagram PRISMA

To define the search strategy and selection process, predefined inclusion and exclusion criteria were applied. Titles, abstracts, and full-text articles were independently screened, and studies that did not meet the eligibility criteria were excluded (figure 2). The guiding PICO question used in the review is presented in figure 3.

PICO:
P: Patients who have received multiple transfusions and developed irregular erythrocyte alloantibodies
I: Immunohematologic techniques used for the detection and characterization of irregular alloantibodies.
C: Comparison between different immunohematologic techniques available over time.
O: Evaluation of the diagnostic performance and clinical utility of the techniques to ensure transfusion safety.
Question:
In chronically transfused patients with a history of alloimmunization, which immunohematologic techniques enable the accurate detection and characterization of multiple simultaneous irregular alloantibodies, thereby reducing the risk of post-transfusion hemolytic reactions?

Figure 3. PICO

RESULTS AND DISCUSSION

A total of thirteen studies met the inclusion criteria, all involving patient populations with a history of multiple transfusions and confirmed alloimmunization. Across these studies, variability was observed in the frequency and distribution of irregular alloantibodies, as well as in the diagnostic performance of the immunohematologic techniques used for their detection and identification.

The most common irregular antibodies were mainly associated with the Rh system, particularly *Anti-E* and *Anti-C*, and with the Kell system, predominantly *Anti-K*. Antibodies from the Kidd *(Anti-Jka)* and Duffy *(Anti-Fyb)* systems were identified less frequently. Combined antibody patterns were also observed, maintaining the predominance of the Rh and Kell systems.

The most prevalent alloantibodies belonged to the Rh and Kell systems, with Anti-E, Anti-K, and Anti-C being the most prominent. These findings are consistent with those reported by Bueno et al., who observed a higher prevalence of Anti-E and Anti-K in patients receiving multiple transfusions, underscoring the high immunogenicity of these antigens.[28] Similarly, Dinić et al. reported that the Rh and Kell systems account for the majority of clinically significant alloimmunizations, reinforcing the need to consider extended red cell compatibility for the C, c, E, e, and K antigens.[29]

The most recurrent antibody combinations identified, such as Anti-D + Anti-C and Anti-E + Anti-Jka, are consistent with the patterns described by Dinić et al., who reported that the coexistence of Rh and Kidd antibodies is associated with progressive alloimmunization, increasing the risk of post-transfusion hemolytic reactions and complicating the selection of compatible units. This serological profile reflects the immune system's capacity to develop a progressive polyclonal response following repeated exposure to different erythrocyte antigens.[29]

The comparison of the immunohematologic techniques revealed marked differences in sensitivity and detection capacity. The tube test, although historically used, shows lower sensitivity and high variability depending on operator expertise.[16] In contrast, column agglutination (CAT) improves standardization and interpretation; however, it may fail to detect low-affinity alloantibodies.[22] Blomme et al. demonstrated that solid-phase red cell adherence (SPRCA) provides superior sensitivity, enabling the detection of weak or masked alloantibodies that may not be identified by CAT or tube methods. This finding was also reported by Cupaiolo et al., particularly in antibodies of the Kidd system, where SPRCA showed significantly greater diagnostic performance.[30,31]

Antibody titers are a key parameter for estimating the clinical risk associated with alloimmunization.[18] Felimban et al. reported that high titers correlate with greater immunologic memory activation and an increased risk of hemolysis during subsequent transfusions, underscoring the importance of continuous monitoring.[32] However, the availability of systematic titration and advanced immunohematologic techniques may be limited in resource-constrained settings. In these scenarios, stepwise diagnostic algorithms become crucial, using CAT as an initial screening method and SPRCA for confirmation and complex antibody identification.[19]

Finally, the implementation of extended compatibility and the use of highly sensitive techniques are essential to ensure transfusion safety, particularly in patients with chronic conditions or prolonged transfusion exposure.[22] The development of phenotyped donor registries and the progressive automation of immunohematologic procedures represent key strategies for improving traceability and ensuring safer and more personalized transfusion practices.[19]

Table 1. Reported findings on erythrocyte alloantibodies and immunohematological detection methods

Study	Population	Immunohematologic Techniques	Irregular Alloantibodies	Relevant Data
Natukunda et al., 2021[15]	311 patients	Three-cell homemade red cell panel expressing C, c, E, e, K, Fya, Fyb, Jkb, S, and s antigens.	Alloantibodies were detected in 27 patients, without specifying the identified antibodies.	•Economical and accessible alternative. •Lower sensitivity compared to commercial panels. •Does not identify Jka, k, M, and N antigens.
Soni-Trinidad et al., 2022 [16]	29-year-old female with beta-thalassemia minor and a history of multiple transfusions during two previous pregnancies.	Conventional tube technique using commercial identification panels.	One alloantibody detected. *Anti-E*	•Alloimmunization confirmed secondary to multiple transfusions during pregnancy. •Extended phenotyping allowed identification of the implicated antigen. •Highlights the relevance of alloantibody screening in pregnant women with hemoglobinopathies.
Aboderin F., et al.,2025 [17]	100 patients: 50 with sickle cell disease who had received ≥2 transfusions, and 50 with sickle cell disease but minimal transfusion history.	Conventional tube technique using commercial antibody identification panels (NHSBT, PR144).	Alloantibodies detected in 50 patients, with *Anti-K* and *Anti-Jka* being the most frequent, followed by *Anti-Fya* and *Anti-M*. Less frequent Rh antibodies included *Anti-C* and *Anti-E*.	•High frequency of alloimmunization in chronically transfused patients. •Antibodies directed against highly immunogenic antigens (Kell, Kidd, Duffy). •Extended phenotype matching is required in high-transfusion-exposure populations.
Faisal I., et al., 2024 [18]	200 patients ≥14 years from medical wards, hemodialysis, obstetrics, and neonatal/prenatal units, most with repeated transfusion history.	Conventional tube technique using homemade red blood cells with the Indirect Antiglobulin Test (IAT). •Alloantibody titration performed on IAT-positive plasma samples. •Extended phenotyping performed in IAT-positive patients for full Rh (C, c, E, e) and Kell antigens using column agglutination. •Antibody identification using the conventional tube technique with a 10-cell commercial panel (NHSBT, PR146).	Alloantibodies detected in 6 patients, mainly *Anti-Jkb* and combinations of *Anti-Jkb* + *Anti-K*, *Anti-Jkb* + *Anti-Fya*, and *Anti-Jkb* + *Anti-Fyb*. Less frequent: *Anti-K*.	•Clinically significant alloantibodies directed against Kidd and Duffy antigens. •Alloimmunization associated with transfusion of more than four units and with a history of pregnancy loss. •Recommendation for systematic alloantibody screening and extended compatibility in women of childbearing age.
Sachan et al., 2020 [19]	64-year-old female with severe liver disease related to hepatitis C, referred for liver transplant surgery.	•Conventional tube Indirect Antiglobulin Test (IAT) using a 3-cell commercial screening panel (ID-Diacell, Bio-Rad). •Antibody identification with conventional tube technique using an 11-cell commercial panel (ID-Diapanel, Bio-Rad). •Extended phenotyping using column agglutination for Rh + Kell (Bio-Rad). •Antibody titration using tube method.	Three alloantibodies were detected: Anti-C Anti-e Anti-K	•More than 800 donor units were screened to obtain only 5 compatible units. •Inter-institutional coordination was required to ensure safe transfusion. •Highlights the importance of donor registries with rare phenotypes. •Emphasizes the need for early transfusion planning in highly immunized patients.

Conrath S et al., 2021[20]	451 patients with sickle cell disease.	•Indirect Antiglobulin Test (IAT) using column agglutination with a 3-cell screening panel. •Antibody identification using column agglutination with a 15-cell commercial identification panel. •Enzyme treatment techniques (papain and/or trypsin) applied when necessary.	Alloantibodies were detected in 134 patients. The most frequent were anti-Lea, followed by anti-M, anti-S, and anti-Leb. Less frequent antibodies included anti-Fya, anti-C, and anti-E, with occasional detection of anti-K.	•High rate of alloimmunization in sickle cell disease due to prolonged transfusion exposure. • Progressive increase in alloantibody formation with increasing number of transfusions. •Implementation of transfusion strategies using leukoreduced RBC units recommended. •Extended phenotyping for D, C/c, E/e, K, Duffy, Kidd, and MNS antigens is advised.
Yadav et al., 2023 [21]	255 patients with beta-thalassemia.	•Indirect Antiglobulin Test (IAT) using column agglutination with a 3-cell commercial screening panel (ID-Diacell, Bio-Rad). •Antibody identification using column agglutination with an 11-cell commercial panel (ID-Diapanel, Bio-Rad).	Alloantibodies were detected in 17 patients, with Anti-K and *Anti*-E being the most frequent. Less frequent combinations observed included: *Anti-D* + *Anti-C*, *Anti-E* + *Anti-K*, and isolated cases of *Anti-Jka* and *Anti-Jkb*.	•Alloimmunization was associated with the total number of transfused units and short transfusion intervals. •Extended Rh and Kell compatibility is recommended in patients with thalassemia requiring repeated transfusions. •Highlights the need for periodic serologic monitoring to prevent hemolytic reactions.
Contelli et al., 2024 [22]	201 patients with hematologic cancer, hemoglobinopathies, or chronic kidney disease with previous irregular alloantibody screening	•Indirect Antiglobulin Test (IAT) using column agglutination with a 3-cell commercial screening panel (ID-Diacell, Bio-Rad). •Antibody identification using column agglutination with an 11-cell commercial identification panel (ID-Diapanel, Bio-Rad).	Multiple alloantibodies were detected in 37 patients. The most frequent combination was Anti-D + Anti-C. Additional associations included antibodies from the Rh and Kell systems, as well as alloantibodies from the Duffy, Kidd, and MNS systems. Cases of coexisting unidentified antibodies were also reported.	•Most cases occurred in women with transfusion and surgical history. •Highlights the importance of detecting and characterizing alloantibodies. •Emphasizes prevention of hemolytic reactions in patients with chronic disease and transfusion dependence.
Wang Y., et al., 2024 [23]	30603 hospitalized pediatric patients (0–14 years).	•Indirect Antiglobulin Test (IAT) using enhanced column agglutination with LISS and a 3-cell commercial screening panel (Ortho Clinical Diagnostics, USA). •Antibody identification using column agglutination with a 16-cell commercial identification panel (Sanquin Reagents, Netherlands).	Alloantibodies were detected in 169 patients, with *Anti-M* being the most frequent, followed by *Anti-E* and *Anti-P1*. Additional low-frequency antibodies were observed in the Rh, Lewis, and Kidd systems.	•Low alloimmunization and autoantibody rates in the pediatric population. •Predominance of naturally occurring MNS system antibodies (Anti-M). •No alloantibody formation in neonates, with progressive development in early childhood. •Recommendation for Rh-E antigen-matched transfusions in pediatric patients.
Calderón W., et al., 2024 [24]	6202 transfused patients at the Regional Blood Fractionation Center of El Oro Province.	•Indirect Antiglobulin Test (IAT) using column agglutination with a 3-cell commercial screening panel (ID-Diacell, Bio-Rad). •Antibody identification using column agglutination with an 11-cell commercial identification panel (ID-Diapanel, Bio-Rad).	Alloantibodies were detected in 56 patients, with *Anti-E* being the most frequent, followed by *Anti-D* and *Anti-Jka*. The remaining alloantibodies were of low frequency, often found in combinations, and a notable percentage showed undetermined specificities.	•Higher alloimmunization frequency in women with transfusion history and older adults. •Need for implementation of extended red cell phenotyping. •Requires systematic pre-transfusion serologic monitoring to prevent hemolytic reactions.

Agrawal S., et al., 2022 [25]	Case 1: 11-year-old girl with jaundice and no prior transfusion history. Case 2: 51-year-old woman with cerebrovascular accident and no prior transfusions. Case 3: 67-year-old man with Chronic Lymphocytic Leukemia and prior transfusion history.	•Antibody detection for the Neo blood group system using solid-phase red cell adherence (SPRCA) with a 4-cell Ready-Screen panel. •Antibody identification using SPRCA with a 16-cell Ready-ID panel. •Antibody identification using column agglutination with an 11-cell commercial identification panel (ID-Diapanel, Bio-Rad). •Antibody identification using the conventional tube technique with a 10-cell commercial identification panel.	Case 1: Warm autoantibodies mimicking *anti-C* and *anti-e*. *Case 2:* Autoantibodies against antigen e. *Case 3:* Alloantibody *anti-E* masked by autoantibody against antigen e.	•Simultaneous presence of autoantibodies and clinically significant alloantibodies. •Difficulty in selecting compatible transfusion units. •Highlights allogenic adsorption as a key technique to differentiate antibody specificities. •Emphasizes the importance of extended Rh phenotyping to accurately define the antibody profile.
Sanders D., 2023 [26]	203 adult patient samples (≥18 years).	•Antibody detection using an automated system (Galileo Echo). •Solid-phase red cell adherence (SPRCA) using a 3-cell Ready-Screen panel. •Indirect Antiglobulin Test (IAT) using column agglutination with commercial panels (Ortho Clinical Diagnostics, USA). •Antibody identification using SPRCA with a 16-cell Ready-ID panel. •Antibody identification using column agglutination with 11-cell commercial identification panels (ID-Diapanel, Bio-Rad). •Proteolytic enzyme (ficin) treatment applied when column agglutination results were discordant.	The most frequent alloantibodies were *anti-E*, followed by *anti-D* and *anti-K*. SPRCA demonstrated higher sensitivity and did not require enzyme treatment, while CAT required enzyme enhancement to reveal weak antibodies.	•SPRCA showed greater sensitivity for detecting weak and early-phase alloantibodies, particularly in the Rh and Kidd systems. •CAT showed lower sensitivity and required ficin treatment in some cases to reveal masked antibodies. •SPRCA improves transfusion safety by enabling earlier detection of clinically significant antibodies. •Highlights the importance of combining techniques to optimize immunohematologic detection.
Devi K., et al., 2025 [27]	8657 patients with transfusion request.	•Antibody screening performed using SPRCA (Solid Phase Red Cell Adherence) with the NEO Iris automated platform and a 3-cell screening panel (Capture-R Ready-Screen). •Antibody identification performed using SPRCA with a 14-cell identification panel (Capture-R Ready-ID).	Irregular alloantibodies were detected in 74 patients, with Anti-E being the most frequent, followed by Anti-D and Anti-Jka. Additional findings included low-frequency antibodies, combined specificities, and occasional cold or undetermined autoantibodies.	•High diagnostic utility of automated SPRCA (NEO Iris, Capture-R) for detecting clinically significant antibodies. •Predominance of antibodies from the Rh and Kidd systems. •The method showed high sensitivity for low-reactivity and complex combination alloantibodies. •Demonstrates superior analytical precision of automated platforms compared to manual techniques. •Supports the implementation of automated immunohematology systems in blood banks.

Table 2. Comparisons of immunohematologic techniques

Immunohematologic Technique	Advantages	Disadvantages
Tube technique using commercial red cell panels	•Low cost and accessible. •Useful in settings with limited resources.	•Low sensitivity. •Incomplete panels due to absence of key antigens. •Requires high technical skill and operator experience.
Tube technique using commercial red cell panels	•Standardized and reproducible. •Detects clinically significant alloantibodies. •Allows the use of enzymes and adsorption procedures.	•Lower sensitivity compared to automated methods. •Subjective interpretation. •Difficulty identifying multiple or masked antibodies.
Column agglutination technique (CAT)	•High sensitivity. •Objective and easy-to-read results. •Suitable for routine screening.	•May fail to detect weak or masked antibodies. •Moderate cost.
Solid-phase red cell adherence (SPRCA)	•Higher sensitivity for weak or multiple alloantibodies. •Does not require enzyme treatment. •Fully automatable.	•High cost. •Not available in all laboratory settings or countries.

Table 3. Irregular antibodies most frequently mentioned in the 13 studies reviewed

Classification	Alloantibodies	Number of Studies Reporting
Individual	*Anti-E* (Rh)	9
Individual	*Anti-K* (Kell)	6
Individual	*Anti-C* (Rh)	6
Individual	*Anti-Lea* (Lewis)	5
Individual	Anti-Jka (Kidd)	5
Individual	*Anti-Fyb* (Duffy)	5
Combination	*Anti-D* + *Anti-C* (Rh)	3
Combination	*Anti-E* + *Anti-Jka* (Rh + Kidd)	3
Combination	*Anti-E* + *Anti-K* (Rh + Kell)	2
Combination	*Anti-C* + *Anti-K* (Rh + Kell)	2

CONCLUSIONS

The most frequent irregular alloantibodies identified were *Anti-E* and *Anti-D* from the Rh system and *Anti-K* from the Kell system, both as isolated antibodies and in combinations such as *Anti-D* with *Anti-C* and *Anti-E* with *Anti-Jka*. These patterns confirm the high immunogenicity of Rh and Kell antigens. The SPRCA technique showed the highest diagnostic sensitivity, particularly in cases involving multiple or masked alloantibodies; however, its cost and limited availability may restrict its routine implementation. In this context, CAT, especially when complemented with enzymatic treatment, remains an accessible and effective option for routine screening. Applying extended erythrocyte compatibility for Rh and Kell antigens in chronically transfused patients is essential to reduce the risk of hemolytic reactions and strengthen transfusion safety.

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